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***In vitro* sensitivity of 30 anaerobic bacterial strains of the human intestinal core microbiota to antibiotics: culture and LC-MS/MS approaches**

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Abstract

We have a vast knowledge on human intestinal microbiota but it can still be regarded incomplete. One of the objectives of scientists using so-called "omics" techniques is to be interested in the consequences that drugs can have on the composition of the intestinal microbiota and inversely. To date, few publications have reported the effects of drugs on the growth of bacteria composing this microbiota using a "culturomics" approach. We focused on antibiotics commonly prescribed for which the only published are the susceptibility of the pathogenic strains and not that of the commensal strains. The aim of our study was to

determine the sensitivity of 30 strains considered to represent the intestinal core microbiota to 8 antibiotics and to study the possible modification of these molecules by bacteria. The 30 bacterial strains were cultured under anaerobic conditions in order to determine their sensitivity to the antibiotics. After 48 hours of culture, the supernatants were also analyzed via UHPLC-MS/MS in order to determine if the antibiotics have been chemically modified. Under the current experimental conditions, cefpodoxime, metronidazole, erythromycin, sulfamethoxazole, trimethoprim and the trimethoprim/sulfamethoxazole combination have little impact on the core microbiota strain growth. On the contrary, moxifloxacin and amoxicillin inhibit the growth of numerous strains of our panel. Using UHPLC-MS/MS analyses, we have shown that some antibiotics can be modified by the bacteria composing the intestinal core microbiome. The bacteria that make up the intestinal microbiota core are impacted by the antibiotics most commonly prescribed in clinics today and inversely.

Keywords

Antibiotics; core gut microbiota; anaerobic; LC-MS/MS, *in vitro*

Introduction

The human gastrointestinal tract is colonized by bacteria, viruses, fungi and protozoa first collectively called the normal flora and more recently the microbiota [1]. This consortium is involved in functions such as host nutrient metabolism, maintenance of the mucosal barrier integrity, immunomodulation and protection against colonization by exogenous potentially pathogenic microorganisms [2]. In recent years, the gut microbiota has been potentially associated with some human health disorders ranging from inflammatory bowel diseases [3], neurological disorders [4] to obesity [5] or cardiovascular diseases [6]. The intestinal bacteria consortium is generally represented by the taxa *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and

Verrucomicrobia, but individual, temporal or spatial variations exist in their distribution [7]. In adults, *Bacteroidetes* and *Firmicutes* are usually predominant, whereas *Verrucomicrobia*, *Actinobacteria* and *Proteobacteria* are in minor proportion [8]. Nevertheless, it has been shown that despite individual variability, there is a core microbiota composed of 57 strains in 90% of individuals and of 18 strains in 100% of individuals [9]. Alterations in the normal microbiota (or dysbiosis) may occur due to changes in diet, radiation or administration of antimicrobial agents, including antibiotics [10]. Nowadays, antimicrobial agents are widely used in human and veterinary health. In 2017, antibiotics use (expressed in daily doses for 1000 inhabitants) was 29.2 for family medicine with 2/3 of prescriptions represented by beta-lactams, penicillin alone or in association. This figure was lower for health institutions that prescribed 2.1 daily doses per 1000 inhabitants [11]. Amoxicillin is the most prescribed antibiotic in France with 20% of prescriptions in hospitals and 41.7% by general practitioners in the community. Amoxicillin coupled with clavulanic acid is the one most given in hospitals, 30.9% against 23.8% in the community. Physicians also prescribe macrolides, tetracyclines, fluoroquinolones and 3rd to 4th generation cephalosporins [12], [11]. It is known for many years that antibiotics used to fight pathogenic bacteria act also on the composition of the intestinal microbiota, leading to adverse effects on normal microbiota [12]. The effect of these molecules on the microbiota depends on numerous factors such as the class of the antibiotic, its administration or its pharmacology. The methods for assessing the impact of antibiotics have mainly consisted in molecular analysis of stools after short term or long-term administration of one or more antimicrobial products [13]. In this context, the aim of this study was to test under *in vitro* anaerobic conditions the effect of eight commonly prescribed antibiotics on 30 bacterial strains of the gut microbiota. In parallel, degradation of these antibiotics by the tested strains has been evaluated using UHPLC-MS/MC methods in order to infer conversely the effect of the microbiota on the molecules tested.

Materials and methods:

Bacterial strains, media and chemicals: The bacterial strains used in this study (Table 1) were purchased from ATCC (American Type Culture Collection) and DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen) or from the University Hospital Center of Poitiers in France. These bacteria were grown anaerobically in absence of free oxygen at 37°C using an anaerobic workstation (Bactron 300, Blanc Labo SA, Switzerland) containing 90% N₂, 5% H₂ and 5% CO₂. The medium used in this study was mGam (Gifu Anaerobic Medium Broth, Modified, Hyserve) [14], allowing the growth of all tested strains, completed with resazurin 0.1% (w/v) as an anaerobiosis indicator. The medium was distributed anaerobically into appropriate containers and finally sterilized by autoclaving at 115 °C for 15 minutes. The pH of the medium was 7.3. The antibiotics (Table 2), purchased from Sigma Aldrich were dissolved and stored in DMSO (Sigma Aldrich) at -20°C.

Sensitivity of the intestinal microbiota strains core to antibiotics: The sensitivity of each strain was determined with respect to 7 antibiotics tested alone and a combination of two antibiotics (Table 2). After an overnight pre-culture at 37°C anaerobically, the OD_{600nm} was adjusted to obtain 1x10⁷ CFU/mL and the bacterial culture was dispatched in 96-well plates. The antibiotics were then tested in triplicate at the concentrations shown in Table 2 and the plates were incubated at 37°C anaerobically. After 48h of incubation, the plates were orbitally shaken at room temperature for 30s, and then the OD_{600nm} was determined using a 96-well microplate reader (TECAN™, Sunrise Remote, Austria). Each bacterial growth was evaluated with or without antibiotic, the antibiotic free condition was used as the control. The percentage of the bacterial growth in the presence of antibiotics was determined compared with its negative control (without antibiotics).

UHPLC-MS/MS method: After 48 hours of incubation, the bacterial cultures were centrifuged (30 min, 4°C; 3000g). The supernatant was then transferred to another 96-well plate and stored at -20°C before the quantity of each antibiotic could be determined by UHPLC-MS/MS. UHPLC-QQQ (ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry) analysis was performed by a 1290 Infinity Binary LC system (Agilent Technologies, Waldbronn, Germany) coupled to a QQQ 5500 mass spectrometer with an ESI Turbo V ion source (SCIEX, Foster City, CA, USA).

Chromatographic separation was performed on C18 column (Poroshell 120 EC-C18, Agilent). The injection volume was 20µl (full loop injection). The mobile phase consisted of two solutions including solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), the column was thermostated in an oven at 35°C and the flow rate was set to 650 µl/min. The chromatographic gradient used for each of the 6 compounds was specific; all details are showed in table S1.

For mass spectrometry analysis, data was acquired using electrospray ionization (ESI) in the positive mode, the ion Spray Voltage was set at 5 500 V. Data was also acquired in negative mode, the ion Spray Voltage was then set at -4 500V. The desolvation in the source was accomplished using the following set parameters: Temperature (TEM) at 600 °C, Ion Source Gas 1 (GS1) at 40 PSI, Ion Source Gas 2 (GS2) at 60 PSI, and Curtain Gas (CUR) at 30 PSI. The specific parameters of multiple reaction monitoring which permit to quantify and monitor the 6 compounds are described in Table S2. Raw data was processed in Sciex Analyst and individual AUC (area under the curve) for each analyte in each sample was determined using the MultiQuant software.

Statistical analyses: Statistical analyses were performed to ensure the significance of the results. The percentage of growth of the different replicates were calculated with respect to the OD_{600nm} and then subjected to the Two-way ANOVA non-parametric test followed by

Šidák multiple comparisons test (GraphPad Prism version 6.01 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com). The % of growth of the controls (without antibiotics) was compared to the % of growth in the presence of antibiotics for each strain. The same analysis was performed for the % of chemical modification from the "below peak area values". The *p*-value used in this test is 0.05. The results are presented in the Table S3 in supplementary files.

Results

In this study, we have tested 7 antibiotics, including 2 in couple and alone, on 30 strains of the core intestinal microbiota in a single medium mGam using 96-wells microplate. The concentrations of antibiotics used in this study were chosen based on the available data from EUCAST and their solubility in DMSO [15]. Metronidazole inhibits the growth of strains belonging to the phyla of *Bacteroidetes* of more than 50% (Figure 1). The inhibited strains were *B. caccae*, *O. splanchnicus*, *P. merdae* and *B. thetaiotaomicron* (> 85%). Moreover, UHPLC-MS/MS results indicate that 18 strains out of 30 of the core intestinal microbiota (6 *Bacteroidetes*, 9 *Firmicutes*, 1 *Proteobacteria* and 2 *Actinobacteria*) modify the metronidazole in proportions greater than 90% irrespective of the phylum. Among these 18 strains, 11 totally modify the molecule. Only two *Firmicutes* (*E. faecalis* and *C. perfringens*) modify the structure of the metronidazole without being affected in their growth. The growth of *B. fragilis*, *B. vulgatus*, *O. splanchnicus* and *P. merdae* is inhibited by this compound although its structure is modified by these strains. No modification of the antibiotic's proportion or structure was observed for only *B. caccae*, *P. distasonis*, *F. nucleatum* and *R. intestinalis* while the growth of these strains is latter being inhibited between 30% and 60%.

The growth of 6 strains in our panel, including 3 *Bacteroidetes*, 2 *Firmicutes* and 1 *Fusobacteria* are inhibited at more than 50% (Figure 1) in presence of cefpodoxime. The

Bacteroidetes had their growth reduced between 60 and 80% compared to the control. In our work, some strains – that are insensitive to cefpodoxime – were capable of modifying this antibiotic at more than 90%. Curiously, the *B. vulgatus* strain was able to modify the antibiotic while it has a growth inhibited of 60%. In this work, the strains capable of modifying the structure of cefpodoxime at more than 90 % are mainly *Bacteroidetes* (5 out of 7). In contrast, the strains that do not modify the structure of the antibiotic, or slightly, are mainly *Firmicutes* (5 out of 9) whose growth is not or only slightly inhibited.

Erythromycin inhibits the growth of 9 strains in our panel to more than 50% (between 50% and 90% inhibition) (Figure 1). The strains impacted belong to the *Bacteroidetes* and *Firmicutes* phyla. *Bacteroidetes* have been the most affected strains by the presence of the antibiotic (6 out of the 10 strains of *Bacteroidetes* of our panel). The disparition of the antibiotic is quite low. Only 3 strains are capable of modifying it in a proportion of 90%. Among these strains, *B. thetaiotaomicron* and *C. perfringens* modify the structure of the antibiotic without being affected in their growth. The strain that modified 95% of erythromycin was *B. caccae*. However, the growth of this strain was inhibited by 70% in the presence of the antibiotic. 9 strains (6 *Firmicutes*, 2 *Actinobacteria* and 1 *Fusobacteria*) of our panel did not or only slightly modified erythromycin (Figure 1).

Moxifloxacin inhibits the growth of 12 strains in our panel by more than 50%. All phyla are concerned including 6 *Bacteroidetes*, 2 *Firmicutes*, 2 *Actinobacteria*, 1 *Fusobacteria* and 1 *Proteobacteria*. Seven strains of our panel, all belonging to the phyla of *Firmicutes*, are not really sensitive to this antibiotic. Furthermore, this compound is slightly modified by the bacteria from our panel. Only *S. parasanguinis* and *C. perfringens* completely modify moxifloxacin. The same applies to *S. parasanguinis*, which is able of completely modify moxifloxacin and whose growth is not affected by the antibiotic. The other strains in our panel do not modify the antibiotic or slightly

We observed that when sulfamethoxazole or trimethoprim were used alone, no significant growth inhibition was noted (Figure 1), except for the *R. torques* strain that has a 40% reduction in growth in the presence of sulfamethoxazole. Up to 90% of sulfamethoxazole, when used alone, is chemically modified by 12 bacterial strains belonging to the phyla of *Bacteroidetes*, *Firmicutes* and *Actinobacteria*. Only *E. lenta* did not modify sulfamethoxazole. Trimethoprim, when tested alone, was modified in more than 90% by a single strain, *F. nucleatum*. In presence of the sulfamethoxazole and trimethoprim couple, the growth of *E. coli* ATCC 8739 is inhibited by 60%. When paired, the sulfamethoxazole is degraded in 90% only by 4 bacterial strains, *S. parasanguinis*, *C. aerofaciens*, *D. formicigenerans* and *R. intestinalis*, instead of 12 strains as previously reported in this study. Trimethoprim is modified by the same strain, *F. nucleatum*, when alone or in combination.

Amoxicillin reduces the growth of 11 bacterial strains from our panel by 50% or more. Growth is inhibited by approximately 80% or more for strains *B. vulgatus*, *E. coli*, *O. splanchnicus*, *C. perfringens* and *C. aerofaciens* (Figure 2). Under our culture conditions, *E. coli* ATCC 8739 is highly sensitive to antibiotics while *B. thetaiotaomicron* was almost non-susceptible to antibiotics with 75% and 25% growth inhibition respectively. Only 5 strains (4 are *Bacteroidetes* and 1 is a *Firmicutes*) from our panel are not affected: *B. fragilis*, *P. merdae*, *B. caccae*, *P. copri* and *C. boltae*. Overall, our results showed growth inhibition with amoxicillin, more or less, for 25 bacterial strains composing the intestinal core microbiota.

Discussion

The aim of our study was to determine the sensitivity of 30 commensal strains of the intestinal core microbiota to 7 antibiotics, including two used alone and in pairs, the most commonly used in France. We showed that metronidazole affected the growth of some strains belonging to the *Bacteroidetes* phyla. These results are in agreement with those of an adult cohort study

which showed that metronidazole treatment decreased *Bacteroidetes* diversity [16] and those described by Narimani *et al.*, in 2016, who showed that the *Bacteroides* that make up the normal intestinal microbiota were susceptible to metronidazole [17]. In addition to its global low activity on the growth of the other strains tested under our conditions, this antibiotic was significantly modified by the bacteria. It was demonstrated by Sousa and his team in 2008 that metronidazole is reduced to N-(2-hydroxyethyl oxamic acid) and acetamide by *C. perfringens* [18]. Our results confirm that this bacterium, but also other strains of the intestinal core microbiota can modify the basic structure of metronidazole (absence of the basic structure of the molecule in 11 supernatants). However, additional analyses will be necessary to know the metabolites produced in our case. In the same way, sulfamethoxazole and trimethoprim have very little impact on the growth of strains of the intestinal core microbiota when used alone or in combination. Overall, we showed that sulfamethoxazole and trimethoprim used alone had no effect on the growth of the bacterial strains. These results are consistent with data from Guerrant and colleagues in 1981, which showed that the administration of trimethoprim to patients had no effect on anaerobic bacteria present in the feces [19]. These antibiotics are always used in combination because it has been demonstrated that the activity was due to a synergy between them [20]. We confirm that when the two antibiotics are tested together, the results are slightly different from the results obtained when the molecules are tested alone. Indeed, in combination, these antibiotics inhibit the growth of 13 strains from our panel. Our *E. coli* strain ATCC 8739 is a part of the panel of growth-inhibited strains which confirms EUCAST data indicating a MIC of <0.5/9.5 mg/L for the trimethoprim/sulfamethoxazole combination for *E. coli* ATCC 25922 [15]. In addition, a study comparing the effects of different antibiotics on anaerobic bacteria in the intestinal microbiota reported low activity of this antibiotic combination against *E. coli* [21]. However, in the presence of trimethoprim alone at 2 mg/L the growth of our *E. coli* strain ATCC 8739 is not inhibited as reported by

EUCAST with an MIC of 1mg/L for the *E. coli* strain ATCC 25922 [15]. The difference in results may be due to the different medium used (EUCAST *E. coli* ATCC 25922 is tested in Mueller Hinton medium), the conditions of exposure to oxygen (under aerobic conditions) and the strain itself. The same phenomenon is observed in the presence of cefpodoxime. Indeed, our *E. coli* strain ATCC 8739 is not sensitive while the EUCAST mentions an MIC of 0.5 mg/L for *E. coli* strain ATCC 25922 [15]. In addition, numerous publications have observed a decrease in the growth of *E. coli* in the presence of cephalosporins [22]. Our other results for cefpodoxime are consistent with antibiotic susceptibility databases which indicate that anaerobic bacteria are not sensitive to cephalosporins, with the exception of *Fusobacterium* and *Prevotella* species [11]. Some of the antibiotic-insensitive strains are able to modify the structure of the antibiotic by more than 90%. This is the case for *C. perfringens* and *C. difficile*. A study conducted in 2006 showed that cefpodoxime could be degraded into 3 compounds depending on humidity and pH conditions [23]. The bacteria could then modify the pH of the medium, leading to a structural modification of the antibiotic (final pH was not studied in this study). Concerning moxifloxacin, *C. perfringens* can also completely modify the structure of this compound without suffering an inhibition of its growth, which is also the case for *S. parasanguinis*. These structural modifications could be an established bacterial mechanism to protect against antibiotics. The insensitivity of our *C. perfringens* strain contrasts with the data of Noel et al. in 2005, who showed a MIC for moxifloxacin between 0.25 mg/L and 1 mg/L for *C. perfringens* [24]. In addition, our *E. coli* strain ATCC 8739 does not react in the same manner (weakly inhibited at 1 mg/L) in the presence of moxifloxacin as the EUCAST strain, *E. coli* ATCC 25922, which is antibiotic sensitive between 0.016 and 0.03 mg/L [15]. These contradictory results could be explained by the different experimental conditions used in the different studies. Overall, 12 strains in our panel have growth-inhibited in the presence of this fluoroquinolone. These results are in agreement with the literature

showing a decrease in bacterial diversity during treatment with moxifloxacin [25,26]. The growth results of our *B. thetaiotaomicron* clinical strain are consistent with EUCAST results indicating insensitivity to moxifloxacin at 2 mg/L [15]. Similar to moxifloxacin, the structure of erythromycin is weakly modified by the bacteria from our panel. Indeed, only 3 bacteria are able to modify the structure of this macrolide to more than 95%. Surprisingly, the basic structure of erythromycin is not found in *B. caccae* supernatants whereas its growth is inhibited by 70% in the presence of the antibiotic. Knowing that erythromycin is degraded in an acidic medium [27], we can hypothesize the strain *B. caccae* used in this study modifies the structure of the antibiotic by acidifying the medium and could therefore produce metabolites that are subsequently toxic to itself. Further studies would be necessary to confirm this hypothesis. Growth inhibition of *B. caccae* and other strains of *Bacteroidetes* phyla is consistent with data in the literature showing a decrease in *Bacteroidetes* and *Clostridia* in feces when this antibiotic is taken [13,28]. The growth of the *E. coli* strain ATCC 8739 used in this study, initially isolated in feces, is not affected in the presence of the antibiotic. These results contradict a study which shows a global disappearance of the bacteria in the feces of patients who had taken erythromycin [29]. However, their study was conducted under aerobic conditions, which may explain the difference in results. In contrast to erythromycin, the data in the literature for amoxicillin indicate an increase in some *Bacteroides* and *Parabacteroides* and a decrease in *Fusobacterium* [13]. Our results on the growth of strains in the presence of this beta-lactam are consistent with this study [13]. However, the *Bacteroidetes* strains *P. distasonis* and *B. vulgatus*, which are inhibited in our study, do not behave as described in this review of the literature on the effect of antibiotics on the composition of the intestinal microbiota [13]. These contradictory results can be explained by another study that showed an increase in certain taxa of *Bacteroidetes* [30]. For amoxicillin, our results for *E. coli* and *B. thetaiotaomicron* are in agreement with those of

EUCAST [15]. Amoxicillin being an unstable molecule [31], it was impossible to perform LC-MS assays after 48 h of culture under our experimental conditions.

With this study, we demonstrated in anaerobic conditions that antibiotics impact the growth of commensal strains of the intestinal microbiota core. We also showed that certain strains are able to modify the structure of antibiotics, mainly metronidazole, without deleterious effect on their growth, suggesting the potential existence of a specific resistance mechanism.

Conclusion

Generally speaking, taking antibiotics is not without risk for the anaerobic bacteria that compose our intestinal core microbiota. We have for the first time shown that, under anaerobically conditions, the tested strains are mainly sensitive to amoxicillin, and moxifloxacin, which are widely-prescribed antibiotics. These molecules act on a large majority of tested strains and can therefore lead to an important dysbiosis. We have also shown that many strains are capable to modify the structure of antibiotics, particularly metronidazole and sulfamethoxazole when coupled with trimethoprim that could lead in some cases, to an inefficiency of the treatment. In addition, the chemical modification of antibiotics by commensal bacteria of the intestinal core microbiota could decrease their *in situ* concentration, which could lead to the appearance of resistance phenomena in pathogenic bacteria. Research on the precise effects of antibiotics on the bacteria of the intestinal microbiota core is still in early stage, because of the difficulty to work with anaerobic conditions. This work could also be conducted with other molecules whose fate and activity on intestinal microbiota is still unknown, despite wide prescriptions.

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Author contribution statement:

F.E., A.C., M.H.R., J.M.B., and M.R. conceived and designed the study. F.E., A.E., S.C., and J.L. conducted the experiments. F.E., A.C., and M.H.R. analysed the results and wrote the paper. All authors reviewed the final manuscript.

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Transparency declarations

No transparency declarations.

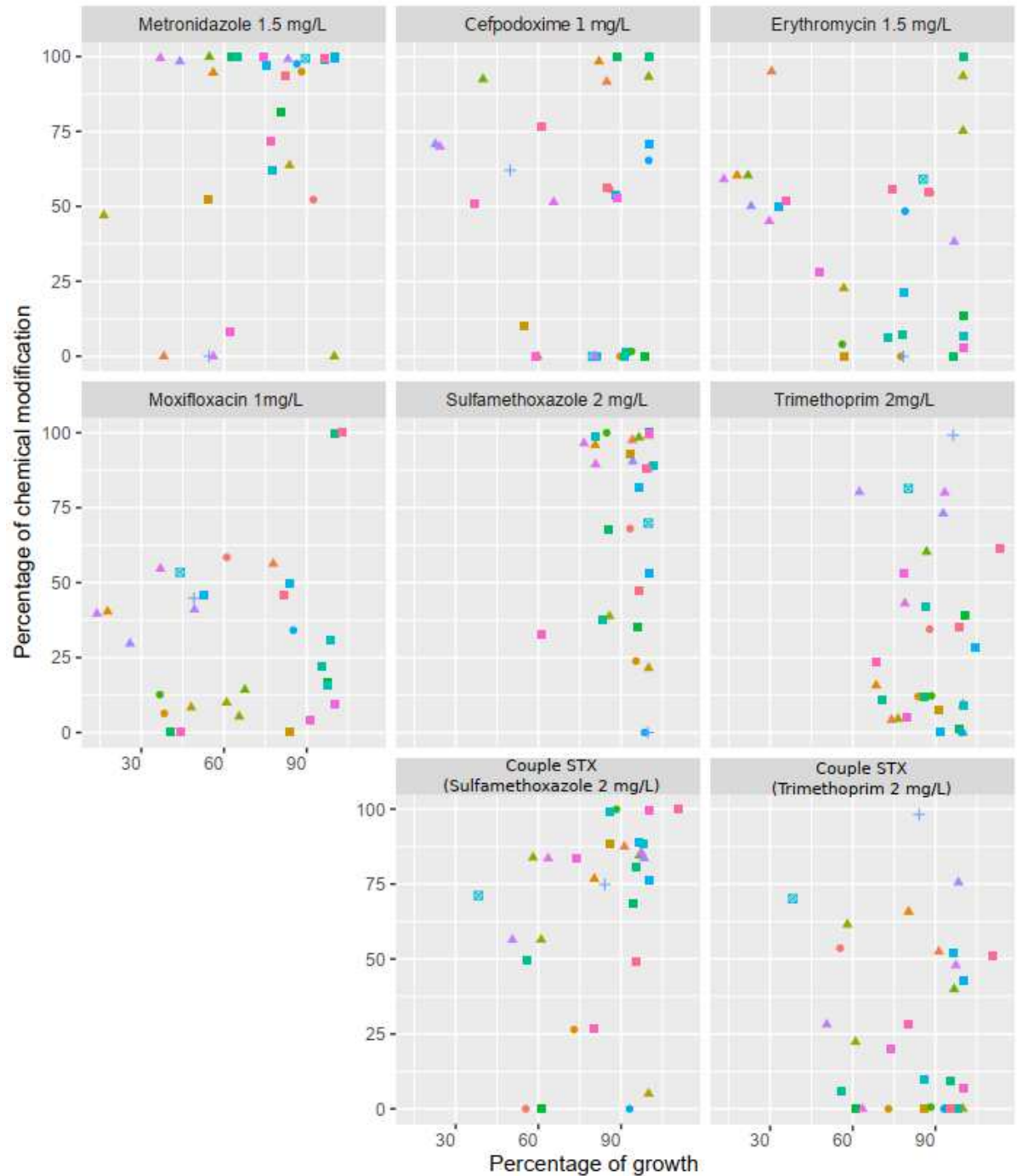
Table 1: Strains used in this study

No accession	Strains	Phyla	Gram
ATCC 8482	<i>Bacteroides vulgatus</i>	<i>Bacteroidetes</i>	Gram -
Clinical strain	<i>Prevotella copri</i>	<i>Bacteroidetes</i>	Gram -
Clinical strain	<i>Bacteroides thetaiotaomicron</i>	<i>Bacteroidetes</i>	Gram -
DSMZ 6597	<i>Bacteroides uniformis</i>	Bacteroidetes	Gram -
DSMZ 2151	<i>Bacteroides fragilis</i>	Bacteroidetes	Gram -
DSMZ 19024	<i>Bacteroides caccae</i>	Bacteroidetes	Gram -
DSMZ 19495	<i>Parabacteroides merdae</i>	Bacteroidetes	Gram -
DSMZ 20701	<i>Parabacteroides distasonis</i>	Bacteroidetes	Gram -
DSMZ 20712	<i>Odoribacter splanchnicus</i>	Bacteroidetes	Gram -
ATCC 8483	<i>Bacteroides ovatus</i>	Bacteroidetes	Gram -
ATCC 8739	<i>Escherichia coli</i>	Proteobacteria	Gram -
DSMZ 15643	<i>Fusobacterium nucleatum</i>	Fusobacterium	Gram -
DSMZ 1402	<i>Clostridium ramosum</i>	Firmicutes	Gram +
DSMZ 14610	<i>Roseburia intestinalis</i>	Firmicutes	Gram +
DSMZ 2008	<i>Veillonella parvula</i>	Firmicutes	Gram +
DSMZ 15670	<i>Clostridium bolteae</i>	Firmicutes	Gram +
DSMZ 11782	<i>Clostridium perfringens</i>	Firmicutes	Gram +
DSMZ 2544	<i>Clostridium saccharolyticum</i>	Firmicutes	Gram +
DSMZ 25238	<i>Blautia obeum</i>	Firmicutes	Gram +
DSMZ 6778	<i>Streptococcus parasanguinis</i>	Firmicutes	Gram +
DSMZ 3376	<i>Eubacterium eligens</i>	Firmicutes	Gram +
DSMZ 3992	<i>Dorea formicigenerans</i>	Firmicutes	Gram +
DSMZ 27543	<i>Clostridioides difficile</i>	Firmicutes	Gram +
ATCC 19433	<i>Enterococcus faecalis</i>	Firmicutes	Gram +

ATCC 27756	<i>Ruminococcus torques</i>	Firmicutes	Gram +
ATCC 29149	<i>Ruminococcus gnavus</i>	Firmicutes	Gram +
DSMZ 20219	<i>Bifidobacterium longum</i>	Actinobacteria	Gram +
DSMZ 20083	<i>Bifidobacterium adolescentis</i>	Actinobacteria	Gram +
DSMZ 3979	<i>Collinsella aerofaciens</i>	Actinobacteria	Gram +
DZMZ 2243	<i>Eggerthella lenta</i>	Actinobacteria	Gram +

Table 2: Antibiotic used in this study

Antibiotics	Concentrations (mg/L)	Family
Metronidazole (MTR)	1.5 mg/L	Nitroimidazole
Cefpodoxime (CPD)	1 mg/L	Beta-lactam
Erythromycin (ERY)	1.5 mg/L	Macrolides
Moxifloxacin (MXF)	1 mg/L	Fluoroquinolone
Trimethoprim (TMP)	2 mg/L	Diaminopyrimidines
Sulfamethoxazole (SMX)	2 mg/L	Sulfonamides
Trimethoprim / Sulfamethoxazole (STX)	2 / 2 mg/L	Diaminopyrimidines + Sulfonamides
Amoxicillin (AMO)	2 mg/L	Beta-lactam



Phyla

- ▲ Bacteroidetes
- Actinobacteria
- + Fusobacteria
- Firmicutes
- ⊠ Proteobacteria

Bacteria

- | | |
|--|--------------------------------------|
| ▲ <i>B. ovatus</i> ATCC 8483 | ■ <i>B. obeum</i> DSM 25238 |
| ▲ <i>B. thetaiotaomicron</i> clinical strain | ■ <i>C. boltae</i> DSM 15670 |
| ▲ <i>B. uniformis</i> DSM 6597 | ■ <i>C. difficile</i> DSM 27543 |
| ▲ <i>B. vulgatus</i> ATCC 8482 | ■ <i>C. perfringens</i> DSM 11782 |
| ▲ <i>B. caccae</i> DSM 19024 | ■ <i>C. ramosum</i> DSM 1402 |
| ▲ <i>B. fragilis</i> DSM 2151 | ■ <i>C. saccharolyticum</i> DSM 2544 |
| ▲ <i>O. splanchnicus</i> DSM 20712 | ■ <i>D. formicigenerans</i> DSM 3992 |
| ▲ <i>P. copri</i> clinical strain | ■ <i>E. eligens</i> DSM 3376 |
| ▲ <i>P. distasonis</i> DSM 20701 | ■ <i>E. faecalis</i> ATCC 19433 |
| ▲ <i>P. merdae</i> DSM 19495 | ■ <i>R. gnavus</i> ATCC 29149 |
| ● <i>B. adolescentis</i> DSM 20083 | ■ <i>R. intestinalis</i> DSM 14610 |
| ● <i>B. longum</i> DSM 20219 | ■ <i>R. torques</i> ATCC 27756 |
| ● <i>C. aerofaciens</i> DSM 3979 | ■ <i>S. parasanguinis</i> DSM 6778 |
| ● <i>E. lenta</i> DSM 2243 | ■ <i>V. parvula</i> DSM 2008 |
| + <i>F. nucleatum</i> DSM 15643 | ⊠ <i>E. coli</i> ATCC 8739 |

Figure 1: Biplots showing the percentage of bacterial growth of intestinal core microbiota strains versus the percentage of antibiotics

modification. The percentage of growth was calculated in relation to the control cultures of each strain in the absence of antibiotics after 48h of culture in mGam medium under anaerobic conditions and after reading of OD_{600 nm}. The percentage of modification was determined by UHPLC-MS/MS in relation to the control samples. Growth data are identical for STX (Trimethoprim 2 mg/L) and STX (Sulfamethoxazole 2 mg/L) combination, only the degradation data are different. All experiments were carried out in triplicate.

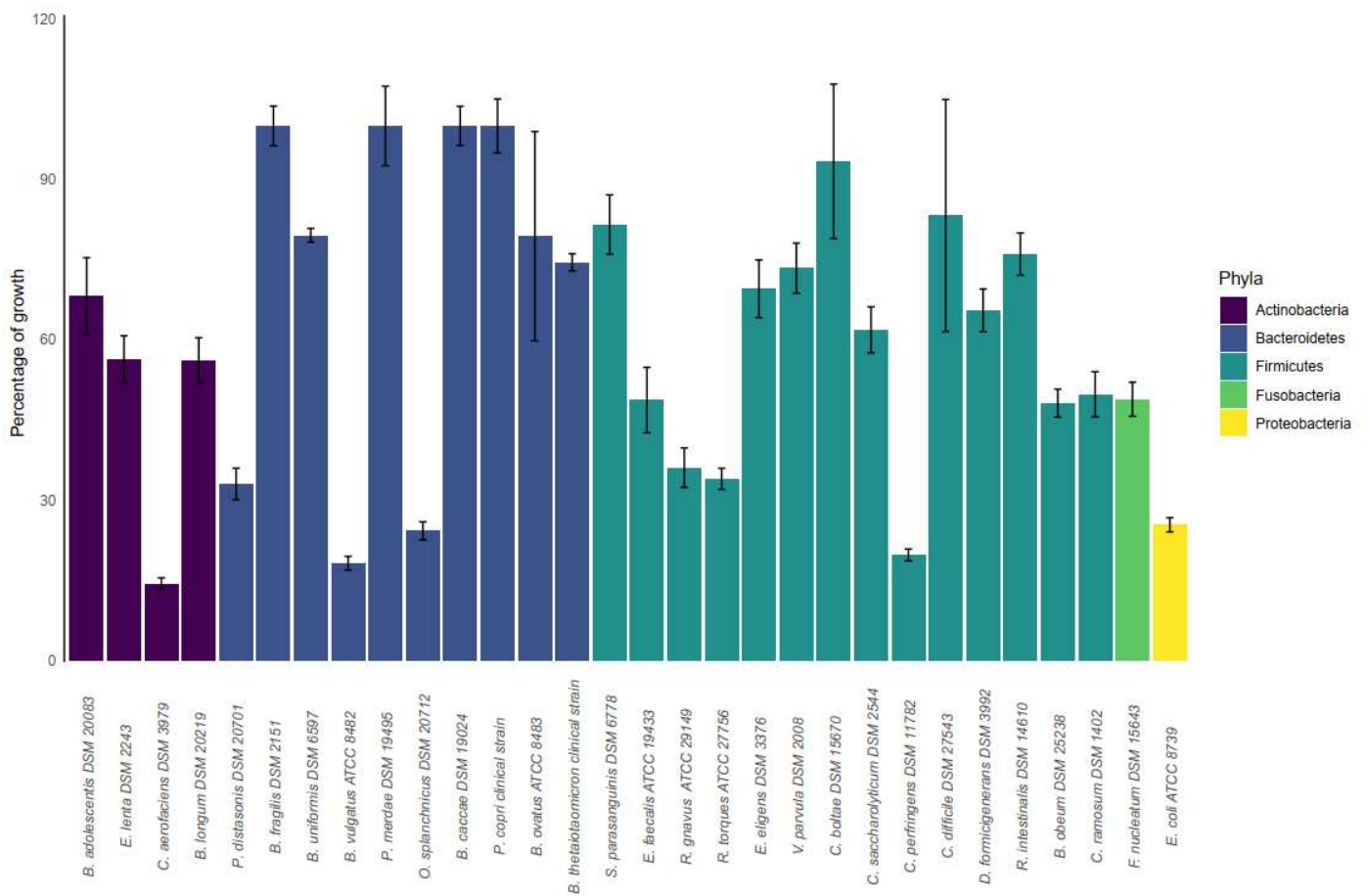


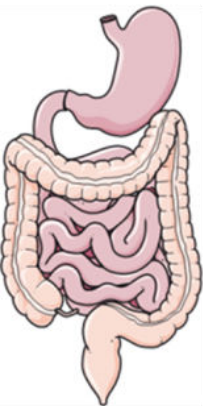
Figure 2: Bacterial growth in the presence of amoxicillin (2mg/L). The bacterial strains were cultured in mGam medium in the presence of amoxicillin at 2 mg/L or not (control). The percentage of growth was calculated relative to the control cultures of each strain without antibiotics after 48 hours of anaerobic culture. All experiments were carried out in triplicate.

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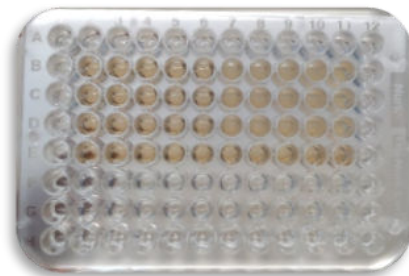
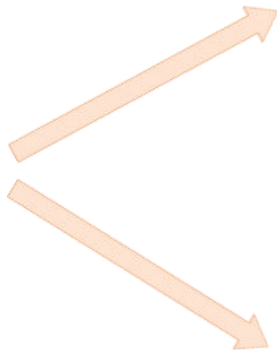
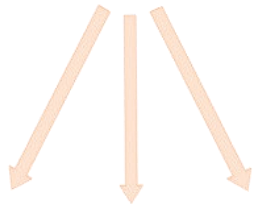
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Sensitivity



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